Anti-IRF3 Antibody [SD2062]

ET1612-14

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 47 kDa
Clone number:	SD2062

Description: Interferon regulatory factor-1 (IRF-1) and IRF-2 have been identified as novel DNA-binding factors that function as regulators of both type I interferon (interferon-α and β) and interferon-inducible genes. The two factors are structurally related, particularly in their N-terminal regions, which confer DNA binding specificity. In addition, both bind to the same sequence within the promoters of interferon-α and interferon-β genes. IRF-1 functions as an activator of interferon transcription, while IRF-2 binds to the same cis elements and represses IRF-1 action. IRF-1 and IRF-2 have been reported to act in a mutually antagonistic manner in regulating cell growth; overexpression of the repressor IRF-2 leads to cell transformation while concomitant overexpression of IRF-1 causes reversion. IRF-1 and IRF-2 are members of a larger family of DNA binding proteins that includes IRF-3, IRF-4, IRF-5, IRF-6, IRF-7, ISGF-3γ p48 and IFN consensus sequence-binding protein (ICSBP).

Immunogen: Synthetic peptide within Human IRF3 aa 71-120 / 427.

Positive control: HeLa cell lysate, Jurkat cell lysate, MCF7 cell lysate, A549 cell lysate, THP-1 cell lysate, Hela-si NT cell lysate, Hela-si IRF3 cell lysate, RAW264.7 cell lysate, mouse spleen tissue lysate, mouse liver tissue lysate, Hela, MCF-7, PANC-1, human breast carcinoma tissue, human kidney tissue, human pancreas tissue, human tonsil tissue, mouse colon tissue, mouse testis tissue, mouse heart tissue, rat brain tissue, rat colon tissue, rat heart tissue, rat spleen tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q14653 Human | P70671 Mouse

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:2,000
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

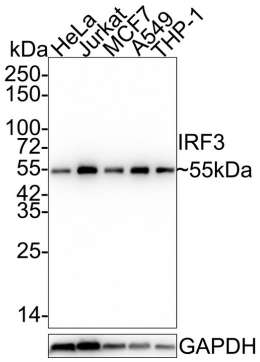


Fig1: Western blot analysis of IRF3 on different lysates with Rabbit anti-IRF3 antibody (ET1612-14) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: Jurkat cell lysate (15 µg/Lane)
Lane 3: MCF7 cell lysate (15 µg/Lane)
Lane 4: A549 cell lysate (15 µg/Lane)
Lane 5: THP-1 cell lysate (15 µg/Lane)

Predicted band size: 47 kDa
Observed band size: 55 kDa

Exposure time: 43 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1612-14) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

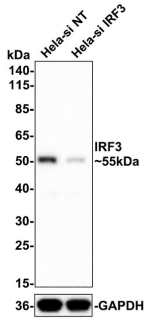


Fig2: Western blot analysis of IRF3 on different lysates with Rabbit anti-IRF3 antibody (ET1612-14) at 1/5,000 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si IRF3 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 47 kDa
Observed band size: 55 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1612-14, 1/5,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

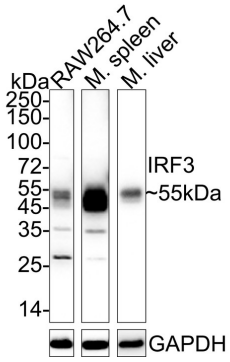


Fig3: Western blot analysis of IRF3 on different lysates with Rabbit anti-IRF3 antibody (ET1612-14) at 1/5,000 dilution.

Lane 1: RAW264.7 cell lysate (20 µg/Lane)
Lane 2: Mouse spleen tissue lysate (40 µg/Lane)
Lane 3: Mouse liver tissue lysate (40 µg/Lane)

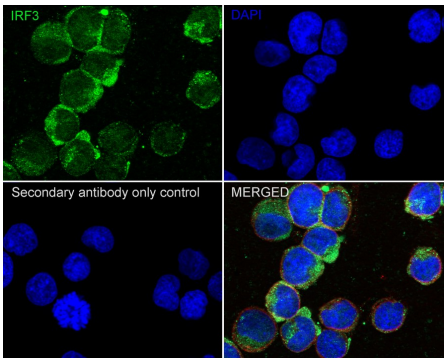
Predicted band size: 47 kDa
Observed band size: 55 kDa

Exposure time: 10 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1612-14) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig4: Immunocytochemistry analysis of Jurkat cells labeling IRF3 with Rabbit anti-IRF3 antibody (ET1612-14) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-IRF3 antibody (ET1612-14) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

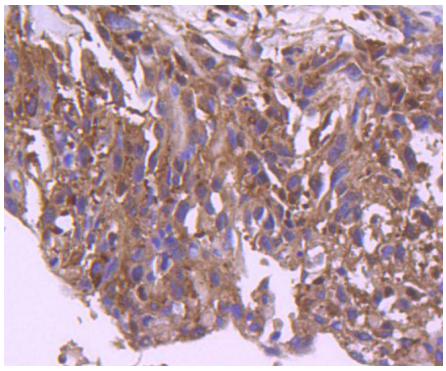


Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-IRF3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

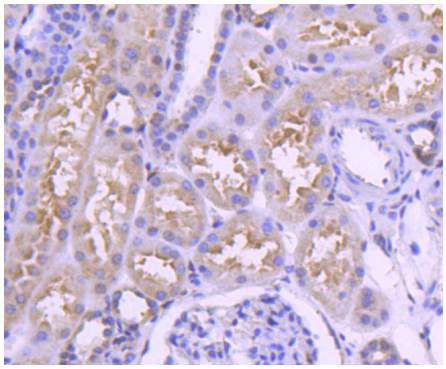


Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-IRF3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

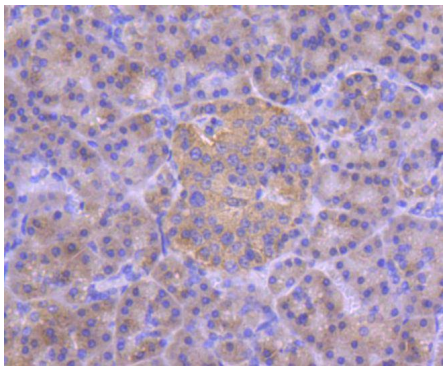


Fig7: Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-IRF3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

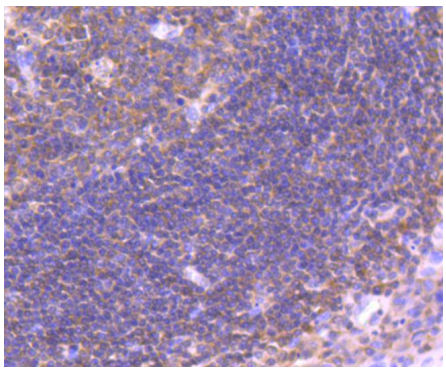


Fig8: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-IRF3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

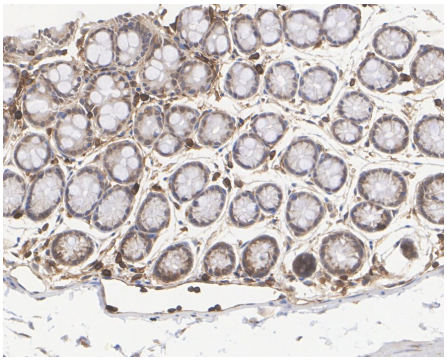


Fig9: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-IRF3 antibody (ET1612-14) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

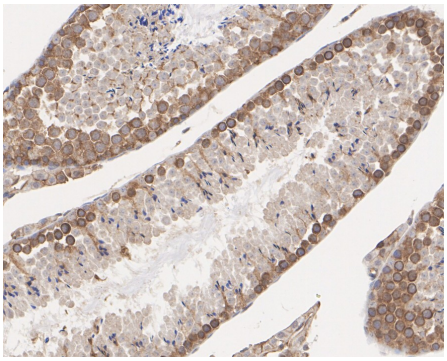


Fig10: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-IRF3 antibody (ET1612-14) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

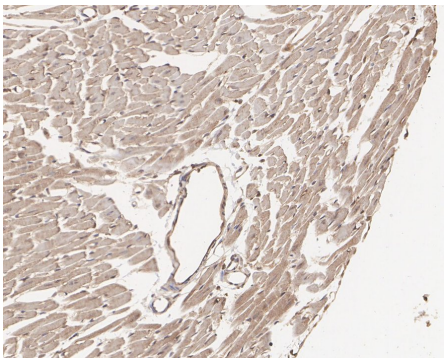


Fig11: Immunohistochemical analysis of paraffin-embedded mouse heart tissue with Rabbit anti-IRF3 antibody (ET1612-14) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

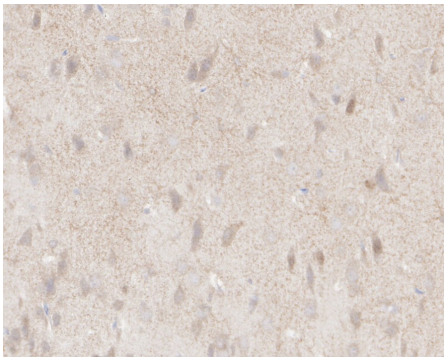


Fig12: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-IRF3 antibody (ET1612-14) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

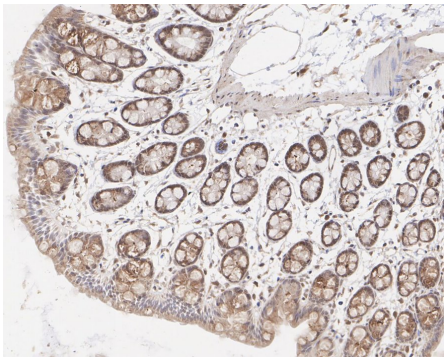


Fig13: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-IRF3 antibody (ET1612-14) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

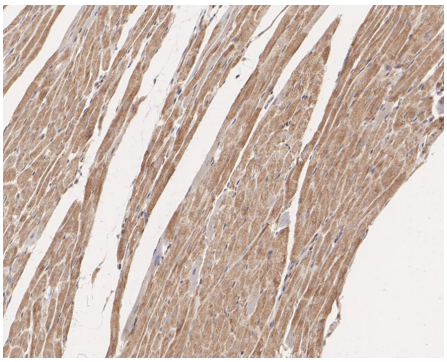


Fig14: Immunohistochemical analysis of paraffin-embedded rat heart tissue with Rabbit anti-IRF3 antibody (ET1612-14) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

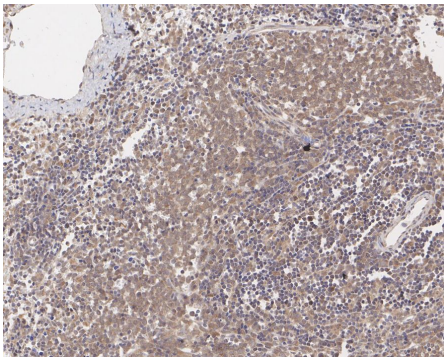


Fig15: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-IRF3 antibody (ET1612-14) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1612-14) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

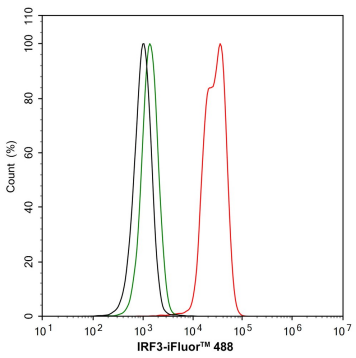


Fig16: Flow cytometric analysis of Jurkat cells labeling IRF3.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1612-14, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

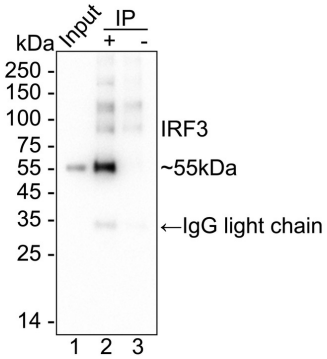


Fig17: IRF3 was immunoprecipitated from 0.2 mg Jurkat cell lysate with ET1612-14 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1612-14 at 1/5,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Jurkat cell lysate (input)
Lane 2: ET1612-14 IP in Jurkat cell lysate
Lane 3: Rabbit IgG instead of ET1612-14 in Jurkat cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 9 seconds; ECL: K1801

Background References

1. Zhou Z et al. TRIM14 is a mitochondrial adaptor that facilitates retinoic acid-inducible gene-I-like receptor-mediated innate immune response. Proc Natl Acad Sci U S A 111:E245-54 (2014).
2. Kolokoltsova OA et al. RIG-I enhanced interferon independent apoptosis upon Junin virus infection. PLoS One 9:e99610 (2014).